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Matrix Metalloproteinases: Role In Arthritis

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1. ABSTRACT

The irreversible destruction of the cartilage, tendon, and bone that comprise synovial joints is the hallmark of both rheumatoid arthritis (RA) and osteoarthritis (OA). While cartilage is made up of proteoglycans and type II collagen, tendon and bone are composed primarily of type I collagen. RA is an autoimmune disease afflicting numerous joints throughout the body; in contrast, OA develops in a small number of joints, usually resulting from chronic overuse or injury. In both diseases, inflammatory cytokines such as interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha) stimulate the production of matrix metalloproteinases (MMPs), enzymes that can degrade all components of the extracellular matrix. The collagenases, MMP-1 and MMP-13, have predominant roles in RA and OA because they are rate limiting in the process of collagen degradation. MMP-1 is produced primarily by the synovial cells that line the joints, and MMP-13 is a product of the chondrocytes that reside in the cartilage. In addition to collagen, MMP-13 also degrades the proteoglycan molecule, aggrecan, giving it a dual role in matrix destruction. Expression of other MMPs such as MMP-2, MMP-3 and MMP-9, is also elevated in arthritis and these enzymes degrade non-collagen matrix components of the joints. Significant effort has been expended in attempts to design effective inhibitors of MMP activity and/or synthesis with the goal of curbing connective tissues destruction within the joints. To date, however, no effective clinical inhibitors exist. Increasing our knowledge of the crystal structures of these enzymes and of the signal transduction pathways and molecular mechanisms that control MMP gene expression may provide new opportunities for the development of therapeutics to prevent the joint destruction seen in arthritis.

2. INTRODUCTION

The two major arthritic diseases are rheumatoid arthritis (RA) and osteoarthritis (OA) (1). RA is an inflammatory and autoimmune disorder, affecting about 1 percent of the world's population(2). OA, on the other hand, is the most common of all joint diseases, and one of the most frequent causes of physical disability. The prevalence of OA increases with age, affecting 80% of people over age 65, and 135 million people worldwide (3). RA is a systemic disease, affecting multiple joints throughout the body, while OA is usually localized to one or two joints that have been subjected to chronic use or injury. Women are two to three times more likely to be afflicted with RA than men, and the genetic marker HLA-DR4 predisposes individuals to the disease. In RA the body's immune system triggers a self-directed inflammatory/immunological cascade that culminates in joint destruction. The pathogenesis of RA originates in the synovial tissues adjacent to the joints and then spreads to the cartilage, while OA begins in the cartilage, and in the advanced stages spreads to include the synovial tissues surrounding the joints (Figures 1 and 2).

The common thread linking RA and OA is irreversible destruction of the cartilage, tendon and bone in the affected joints. The progressive degradation of these connective tissues seen in both diseases leads to pain, loss of joint function, and a substantial decrease in quality of life. In this review, we will examine the mechanisms by which this connective tissue destruction occurs and the strategies that have been employed to prevent or reduce it.

3. CARTILAGE IN HEALTH AND DISEASE

Healthy articular cartilage is composed of a highly organized network of collagen and proteoglycans

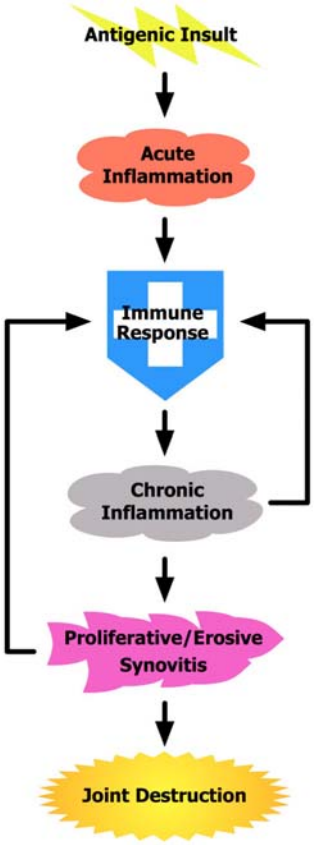

Figure 1. Pathogenesis of rheumatoid arthritis.

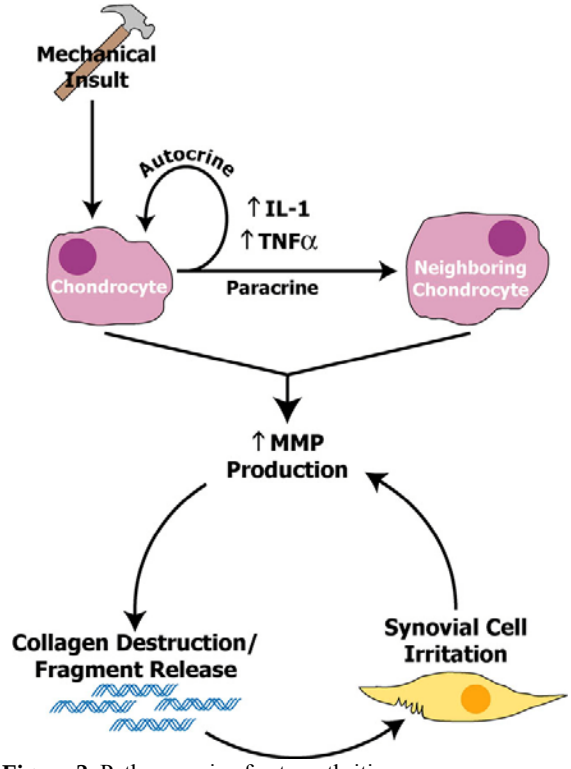

Figure 2. Pathogenesis of osteoarthritis.

(4). The proteoglycans are very large molecules (molecular weight of several hundred thousand), made up of a protein backbone covalently linked to copolymer chains of negatively charged disaccharide carbohydrates, called glycosaminoglycans (GAGs), (e.g., keratan sulfate, chondroitin sulfate, or dermatan sulfate). Aggrecan is the most predominant of these proteoglycans, and is a key component of cartilage. The proteoglycans then non-covalently associate with hyaluronic acid, made up of repeating glucuronic acid and N-acetylglucosamine disaccharides, to create a huge proteoglycan aggregate. These aggregates attract water to their negatively charged GAGs leading to significant hydration and swelling of the tissue. This architecture creates a low coefficient of friction on the joint surfaces and gives the cartilage protective resiliency.

The structural rigidity of cartilage is conferred by collagen fibrils, made up primarily of type II collagen with more minor contributions from type IX and XI collagen (5). Type II collagen is almost unique to cartilage. It is made up of three alpha 1 (type II) chains, and unfortunately, when this collagen is destroyed, it is replaced with a type I collagen fibro-cartilage that does not have the same functional properties as type II collagen. In normal physiology, the synthesis and degradation of the matrix proteins in the joint are in equilibrium. However, in RA and OA, there is excessive degradation, leading to progressive loss of matrix proteins and joint integrity (6). Type II collagen and the proteoglycan aggrecan are the two major targets of this degradation, and their loss contributes substantially to the progression of both RA and OA (7, 8).

Although RA and OA culminate in connective tissue destruction, the pathogenesis of these two diseases differs substantially. RA is a systemic autoimmune disease, which is sometimes triggered by an infection (9). The causative agent may be disseminated throughout the body, including the joints. As part of a defense response, inflammatory neutrophils migrate to the site and end up degrading matrix components as they attack the invading organisms. Matrix component fragments are then liberated into the circulation, where they stimulate an autoimmune response in susceptible individuals. Auto-reactive immune cells home to the joints and attack their cognate antigens. Macrophages are then recruited to the joints where they release inflammatory cytokines such as interleukin-1 beta (IL-1 beta) and tumor necrosis factor-alpha (TNF-alpha), along with interleukin-6 and -8, and various growth factors. The cytokines induce expression of matrix metalloproteinases (MMPs), the proteinases largely responsible for the irreversible destruction of cartilage, bone and tendons in the joints. Growth factors in the inflamed synovium drive the proliferation of resident synovial fibroblasts, and these cells, along with the recruited macrophages and immune cells make up a large cellular mass called a pannus. The pannus invades and destroys cartilage, tendon, and bone, and has been likened to a localized malignancy because of its aggressive and invasive behavior.

Originally OA was seen as a disease of passive destruction, in which matrix degeneration was due to the

wear and tear of chronic compression of the joint(10). The chondrocytes were thought to be metabolically quiescent and unable to overcome mechanically induced damage. However, evidence accumulated that chondrocytes were active cells with increased metabolism in OA(11). It became apparent that these cells were major contributors to the pathology of this disease, and that much of the connective tissue degradation was due to proteinases produced by these cells in response to inflammatory stimuli (11).

Biomechanical signaling may initiate the pathology of OA, since there is considerable signaling between chondrocytes and their surrounding matrix (12-14). Signaling through integrin adhesion molecules has been associated with cartilage damage (15, 16), and with the production of proteinases and cartilage components by chondrocytes (17). In addition, chondrocytes produce proinflammatory cytokines such as IL-1 beta, and TNF-alpha (18), thereby creating their own inflammatory environment. These cytokines increase the synthesis of matrix-degrading proteinases. It was noted that the first signs of type II collagen breakdown were immediately adjacent to the edges of chondrocytes, indicating that the chondrocytes in the articular cartilage were degrading the same matrix that they produced (19, 20).

There is also a second route that leads to cartilage destruction in OA. This pathway is thought to be secondary to the initial cartilage breakdown mediated by the neighboring chondrocytes (18) and involves inflamed synovial fibroblasts and infiltrating immune cells, similar to the pannus seen in RA. This pathway develops as cartilage breakdown products are released into the joint fluid and irritate the synovial membranes lining the joint space (18). This synovitis, which is usually mild to moderate but can be as severe as that seen in RA (21-23), provokes the release of inflammatory mediators from synovial tissue and initiates the recruitment of new mononuclear inflammatory cells to joint tissues (24, 25). These arriving cells secrete IL-1 beta and TNF-alpha, which further upregulate the production of proteinases (26, 27). Eventually, a feed-forward loop is created as fragments of cartilage broken down by proteinases produced by the chondroyctes irritate the synovium. This irritation creates a secondary synovitis, with subsequent increases in proteases and inflammatory cytokines from synovial cells and the attraction of additional immune cells.

4. CONNECTIVE TISSUE DEGRADATION IN ARTHRITIS

Matrix proteins can be degraded by four classes of enzymes: cysteine, aspartate- and serine-dependent, and metalloproteinases, each of which is characterized by the amino acid or chemical group in the catalytic site of the enzyme (28). The cysteine and aspartate-dependent proteinases act at low pH, and are primarily intracellular, while metalloproteinases and serine-dependent proteases act extracellularly at neutral pH. While all 4 classes of enzymes can be found in arthritic joints, the metalloproteinases are predominantly responsible for connective tissue destruction. More specifically, two families within the metalloproteinase class, a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) and the matrix metalloproteinases (MMPs), are the major mediators of cartilage destruction in arthritic disease (7-9, 29-31).

Collagen is the most abundant protein in the animal kingdom, accounting for 30% of total body protein (32). As noted, the type II collagen in cartilage and the type I collagen in the adjacent tendons and bone give the joints their structural rigidity. Importantly, since collagen degradation is mediated almost exclusively by the MMPs, the MMPs with collagenolytic ability (collagenases) are rate limiting in collagen degradation. These collagenases make the initial cleavage in the collagen triple helix between Gly775 and Leu776, allowing the collagen chains to unwind. These denatured molecules are then susceptible to attack and further degradation by other MMPs. Concomitantly, the MMPs are also able to degrade non-collagen matrix proteins in cartilage, as can the ADAMTSs.

The ADAMTSs are relative newcomers to our understanding of matrix destruction in arthritis (33). Since the first ADAMTS was discovered in 1997, this proteinase family has grown quickly, and there are now nineteen members. Their enzymatic activities are varied and include cleaving collagen propeptides, inhibiting angiogenesis, and degrading cartilage proteoglycans (33). The aggrecanases (ADAMTS-1, -4, -5, -8, -9, and -15) have demonstrated roles in the pathology of both OA and RA. In early studies, it was found that synovial fluid from patients with arthritis contained aggrecan core protein fragments with the N-terminal sequence Ala-Arg-Gly-Ser, demonstrating that cleavage occurred at the Glu373 - Ala374 bond (34). These same fragments could be found when bovine cartilage explants were stimulated with IL-1 beta (35, 36). Although it had been shown that MMPs could cleave aggrecan (37-40), the MMP cleavage site did not match that of the fragment found in the synovial fluid of arthritic individuals. At the time, Glu373 - Ala374 cleavage activity was attributed to an unidentified ‘aggrecanase’, and this degradative activity has since been identified as ADAMTS-4 and -5, also known as aggrecanase-1 and -2 respectively (41, 42). ADAMTS-5 is constitutively expressed in cartilage, and ADAMTS-4 expression can be induced with IL-1 beta or TNF-alpha treatment (43). The discovery of the ADAMTSs represents a milestone in our understanding of the pathogenesis of OA and RA. ADAMTS-4 and –5 are the primary mediators of aggrecan cleavage in situ and as such, they have significant roles in cartilage degradation (34, 44).

5. MMPs AND CARTILAGE DEGRADATION

Taken together, MMPs can degrade all components of the extracellular matrix (45). MMPs have been divided into 5 categories: 1) the collagenases (MMP-1, -8, -13), 2), which degrade the interstitial collagens (types I, II and III), the gelatinases (MMP-2, -9), which target type IV collagen in basement membrane, 3) the stromelysins (MMP-3, -10, -11), which degrade non-

collagen matrix proteins, 4) the membrane-type MMPs (MMP-14, -15, -16, -17, -24, -25), and 5) a diverse subgroup including MMP-7, -11, -12, -20, and MMP-23.

The collagenases (MMP-1, 8, -13), the gelatinases A and B (MMP-2 and -9 respectively), the stromelysins (MMP-3, -10, -11), the matrilysins (MMP-7, -26), and the MT-MMPs (membrane-type MMPs) are all expressed at low levels in normal joint tissue; however this expression is greatly increased in arthritic joints (46, 47). Each collagenase can cleave the collagen triple helix, although there is some substrate preference. MMP-13 is preferentially expressed by articular chondrocytes and, interestingly, it preferentially cleaves type II collagen. In fact, MMP-13 has five to ten times more activity than MMP-1 on type II collagen, while MMP-1 is more effective against type III collagen, and MMP-8 has greatest activity against type I collagen. Despite these somewhat subtle substrate specificities, it is important to remember that they are all effective in cleaving the collagen triple helix.

The level of MMP-1 expression is often 10-fold higher than the level of MMP-13 expression (48), suggesting that the sheer amount of MMP-1 can overcome its comparative lack of efficiency in degrading type II collagen. MMP-1 and -8 have been localized to the more superficial surface of cartilage, while MMP-13 is found in the deeper layers (49, 50). This distribution pattern may reflect the fact that MMP-1 and MMP-8 are primarily products of synovial cells and neutrophils, respectively, which are adjacent to the cartilage, while MMP-13 expression predominates in the chondrocytes. Once the collagen triple helix has been cleaved by the collagenases, the gelatinases (MMP-2 and -9) can further degrade the denatured collagen/gelatin.

In both RA and OA, the degradation of non-collagen matrix components is mediated, at least in part, by the stromelysins and the matrilysins. The stromelysins have broad specificity against molecules such as fibronectin, elastin, laminin, and aggrecan and MMP-3 (stromelysin–1) activity may be linked specifically to proteoglycan loss (51). In addition, MMP-3 contributes to the activation of proMMP-1 (52), thus giving MMP-3 a dual role in matrix destruction. Matrilysin can also cleave proteoglycans and it is induced by TNF-α and IL-1 beta (53, 54). The MMPs that attack proteoglycan cleave the aggrecan core protein at the Asn341-Phe342 bond in the interglobular domain between G1 and G2 (39, 40). In the matrix, the G1 domain interacts with hyaluronic acid and link protein, and when aggrecan molecules are cleaved, they lose this domain, dissociate from the matrix and diffuse into the synovial fluid where they no longer contribute to cartilage function (36).

Of the six membrane-type matrix metalloproteinases described, only MT1- and MT3-MMP have been documented in the pathogenesis of RA and OA, with MT1-MMP playing the dominant role. MT1-MMP contributes to tissue destruction indirectly and directly. Indirectly, MT1-MMP is expressed in both the superficial and transitional zones of OA cartilage (55) where it activates proMMP-2 (56) and proMMP-13 (57) via proteolytic cleavage, allowing these enzymes to degrade collagen, gelatin, and proteoglycans. More directly, RA fibroblasts and osteoclast-like cells express MT1-MMP. This enzyme has collagenolytic ability, and may mediate resorption of bone (58). The expression pattern of MT3-MMP mimics that of MT1-MMP, suggesting that it may have activities similar to that of MT1-MMP (58, 59). Thus, the profile of MMPs expressed by connective tissues in arthritic joints is sufficient to completely destroy the structural collagens that comprise articular cartilage and the adjacent tendons and bones as well as the non-collagen matrix molecules that contribute to joint integrity and function.

6. REGULATION OF MMP GENE EXPRESSION IN ARTHRITIS

The synthesis of MMPs is tightly regulated at the level of gene expression and in a tissue-specific manner (60). In normal connective tissues, physiologic expression is low, but increases considerably under the pathologic conditions of OA and RA. In arthritic tissues, MMP-2 and MT1-MMP are constitutively expressed, probably because the promoters of these genes do not contain a TATA box (60, 61). In contrast, MMP-1, MMP-3, MMP-9, and MMP-13 are all induced by IL-1 beta and TNF-alpha. The promoters of the MMP genes have been well characterized, and the activator protein 1 (AP-1) binding site at -73bp is a key regulator of MMP transcription. AP-1 is a protein complex composed of c-Fos/c-Jun heterodimers or c-Jun/c-Jun homodimers (60, 61). The MMP-1, MMP-3, MMP-9, and MMP-13 promoters contain this proximal AP-1 site, which is critical for expression. AP-1 proteins also cooperate with other transcription factors, such as Ets proteins that bind to polyoma virus enhancer activator-3 (PEA-3/ETS) sites (60, 61).

There is another important AP-1 site located at –1602 in the MMP-1 promoter (61). This site is adjacent to a single nucleotide polymorphism (SNP) at –1607 bp. This SNP is the presence or absence of an extra guanine, where the sequence 5'-GGAA-3' (2G allele) creates a consensus binding site for the ETS family of transcription factors, while the sequence 5'GAA-3' (1G allele) does not (61). The AP-1 site and the ETS allele cooperate to enhance transcription from the 2G allele. At least one copy of the 2G allele is present in ~75% of individuals and has been associated with the progression of at least seven cancers, as well as periodontal disease and atherosclerosis (60, 61). Its role in arthritis, however, has not been determined.

IL-1 beta and TNF-alpha regulate MMP gene expression through signal transduction pathways, such as those regulated by mitogen-activated protein kinases (MAPKs) (62). The c-Jun N-terminal kinase (JNK) phosphorylates c-Jun to activate a DNA-binding AP-1 complex. In response to inflammatory stimuli, AP-1 also induces the synthesis of c-fos and c-jun mRNAs. The MAPK, p38, also activates factors required for the synthesis of AP-1 and Ets proteins. Thus, the MAPK

pathways are important signaling mediators of inflammatory cytokines and they target the AP-1 and Ets sites in the MMP promoters.

Nuclear factor-kappa B (NF-kB) is another cytokine-induced transcription factor with a critical role in controlling MMP expression in arthritis. NF-kB is a dimer of p50 and p65 subunits (61-63) and is inactive in the cytoplasm when bound to the inhibitor of NF-kB (IkB). When cells are stimulated with IL-1 beta or TNF-alpha, IkB is phosphorylated and targeted for destruction in the proteosome. The p50 and p65 subunits dissociate from IkB and translocate to the nucleus, where they target their binding sites in gene promoters (consensus sequence = GGGACTTTCC) and activate transcription. The promoters of MMP-1, MMP-3, and MMP-9 contain canonical binding sites for NF-kB, and activation of NF-kB is required for transcriptional induction of these genes. In contrast, the promoter of MMP-13 does not contain an NF-kB site, although inhibiting NF-kB blocks induction of MMP-13 by inflammatory cytokines. NF-kB is, therefore, required for transcriptional regulation of MMP-13, but the mechanism is not known. In addition to AP-1 and NF-kB, the transcription factor core binding factor alpha-1 (CBFA-1 also known as Runx-2) is also required for expression of MMP-13 in osteoblasts and chondrocytes, where it confers tissue-specific expression (64, 65).

Transforming growth factor beta (TGF-beta) is another regulator of MMP expression, as well as many other genes. Expressed in arthritic joints, TGF-beta can have both stimulatory and repressive effects (66). TGF-beta signals through the TGF-beta receptor complex and activates Smads, co-factors that translocate to the nucleus and regulate gene expression (67). Smads control the expression of MMPs by binding directly to promoter elements or through interactions with other transcription factors. The promoters of MMP-1 and MMP-3 contain a TGF-beta inhibitory element (TIE) responsible for repression by TGF-beta, while interactions between Smads and AP-1 regulate MMP-13 (61, 68). Importantly, TGF-beta can have differentially affect MMP expression depending on the physiologic state of the cells. For example, MMP-13 is downregulated in chondrocytes adjacent to OA lesions, but upregulated at more distant sites, suggesting that different cytokine environments may influence the effect of TGF-beta (69). In summary, AP-1, Ets, NF-kB, and Smad proteins play important roles in the regulation of MMP gene expression, and modulation of these transcription factors may alter the levels of MMPs and impact disease processes.

Although transcription is the major mechanism regulating MMP gene expression, mRNA stability also plays an important role (60). This is especially true in terms of the cellular responses to TNF-alpha and IL-1 beta. The 3' untranslated region of several MMP genes, including MMP-1, MMP-3, and MMP-13, all prominent contributors to joint destruction in both OA and RA, contains the sequence AUUUA, which destabilizes the mRNA. Thus, under normal conditions, these mRNAs are rapidly turned over. This likely provides a mechanism that ensures that physiologic levels of MMPs remain low. However, when cells are exposed to the inflammatory cytokines, there is a brief transcriptional response, which is accompanied by a substantial increase in mRNA half-life. The AUUUA regions appear to mediate this increase, since mutating these sequences destabilizes the mRNAs (60).

7. INHIBITING MMPs IN ARTHRITIS

7.1. Background

Given the destructive potential of MMPs and their aberrantly high levels of expression in arthritis, abolishing or even reducing these pathologic levels should have substantial clinical benefit. Although efforts to block the destructive activity of MMPs have been ongoing for many years (70-72), there remains no effective MMP inhibitor for treating arthritic diseases (73). Current therapies, such as non-steroidal anti-inflammatory drugs (NSAIDs), may alleviate symptoms by reducing pain and inflammation, but destruction of connective tissues often continues unabated.

Several endogenous proteins can block MMP enzyme activity. One of these, alpha$_2$-macroglobulin (alpha$_2$M), is a large (750 kD) tetrameric glycoprotein found in plasma and produced by the liver (74). It is a general proteinase inhibitor, and it uses a unique mechanism to inhibit proteolytic activity by attacking the proteinase on the "bait" region of alpha$_2$M. Cleaving this region causes a structural change in the inhibitor, trapping the proteinase in the alpha$_2$M molecule (75) thereby neutralizing the enzymatic activity of the proteinase. Although this non-specific proteinase inhibitor is often the first line of defense against pathologic tissue breakdown, its relatively large size precludes it from perfusing into cartilage. Therefore, its role seems largely confined to the inflammatory fluid surrounding the joint (76).

Other physiologic inhibitors of MMPs are the tissue inhibitors of metalloproteinases (TIMPs). In contrast to alpha$_2$M, TIMPs specifically inhibit MMPs (reviewed in (75, 77)), and are produced by the connective tissue cells that make MMPs. TIMP-1, for example, was originally purified from the culture media of human fibroblasts (78). Currently, four family members have been identified (TIMP-1, -2, -3, -4) (77). Inhibiting MMP activity by TIMPs involves non-covalent binding to the target MMP active site with 1:1 stoichiometry. A conserved cysteine residue at position 1 of the TIMP chelates the MMP active site zinc ion and expels the essential water molecule (79). The tertiary structure of all TIMPs is conserved, and therefore, all TIMPs can inhibit all MMPs, with the exception of TIMP-1 and MT1-MMP (80). Due to the fact that TIMPs 1) can block the activity of nearly all MMPs, and 2) are produced by chondrocytes and fibroblasts, the concept of using them as therapeutic agents is appealing. In the pathologic environment of an arthritic joint, the elevated levels of MMPs usually exceed those of the TIMPs (81), rendering the TIMPs rather ineffective. This observation has helped to support the emerging idea of targeted over-expression of TIMPs as a clinical therapy (75, 82). In OA and RA, TIMP-3 is the leading candidate

since it blocks ADAM-17 (83) and the ADAMTS-4, -5 aggrecanases (84) as well as the MMPs. However, in addition to blocking MMP enzyme activity, over-expression of TIMP can have paradoxical effects, such as increased cell growth and invasion, decreased angiogenesis, and increased apoptosis (85-88). Regardless of these limitations, knowledge of the TIMP/MMP crystal structures (89-91) has led to the suggestion of genetically engineering TIMPs so that they are selective for particular MMPs (reviewed by Nagase and Brew (92)).

7.2. Small molecule inhibitors of MMP activity

The development of synthetic matrix metalloproteinase inhibitors (MMPIs) has focused on blocking the proteolytic effects of the activated MMP enzymes, and on preventing activation of the proenzyme (73). A great deal of effort has been expended in the attempt to design small molecule inhibitors that target a specific MMP (93). However, single target inhibitors have yet to be developed.

One group of inhibitors is the peptidomimetics. They have a backbone that mimics the region of collagen surrounding the scissile bond (Gly*Ile-Ala-Gly), which targets the compound to the active site of the MMP. In addition, peptidomimetics have a functional group that chelates the catalytic zinc atom. Chemical groups that bind zinc include the carboxylates, aminocarboxylates, sulfhydryls, phosphoric acid derivatives, and hydroxymates, all of which have been tested for their ability to inhibit MMP activity (94). Hydroxymates have received the most attention, with the batimastat (BB-94) (95, 96) and its orally bioavailable cousin, marimastat (BB-2516) (97, 98) the most thoroughly studied. Although they have an IC50 in the pico- to nanomolar range, they are relatively non-selective, since they inhibit MMP-1, -2, -3, -7, -9, and -12 (99).

Several synthetic MMPIs have been used in human clinical trials, with generally disastrous results (99-101). Specifically, the broad substrate specificity of marimastat and similar inhibitors has probably caused the significant side effects associated with their chronic administration. Most of these initial trials were carried out on patients with cancer, with the goal of preventing tumor progression and metastasis. In phase I trials with escalating doses of drugs, many patients developed a chronic musculoskeletal syndrome characterized by tendonitis and inflammation that presented as joint pain, edema, reduced mobility, and skin discoloration (102, 103). These side effects were eventually ascribed to inhibiting the low levels of MMPs and ADAMTSs needed for the normal physiologic turnover of connective tissue (104). Although these side effects were reversed with brief breaks in drug administration, they limited the doses that were used in later trials (100).

Another group of small molecule inhibitors are the non-peptidomimetic compounds. They also chelate the catalytic zinc ion but are designed to incorporate information about the active site pocket of the MMP they are targeting. This allows for greater specificity and better bioavailability and pharmacokinetics (105). Examples of these inhibitors are AG3340 (Prinomastat®) (106), and BAY 12-9566 (107) were which are selective for MMP-2, and Ro 32-3555 (Trocade®) (45, 108, 109), which targets MMP-1, was thought to be useful in treating arthritis, but has recently been withdrawn from the market (93). Current compounds under clinical investigation for arthritis indications include CPA-926 in phase 2 trials (110), DPC-333 also in phase 2 trials (111), and ONO-4817 in phase 1 trials (112).

A third class of MMPIs is the tetracyclines, which can inhibit MMPs at multiple levels: (a) blocking enzyme activity by zinc chelation, (b) preventing proteolytic activation of the proMMP, and (c) reducing MMP gene expression (113-115). The tetracycline family consists of the traditional antibiotics such as tetracycline and doxycycline, and the chemically modified tetracyclines (CMTs), in which the diethylamino group from the A-ring has been removed, thus eliminating their antibiotic activity (reviewed in (116)). The CMTs have several pharmacologic advantages over traditional tetracyclines, including decreased gastrointestinal toxicity, higher plasma drug concentrations, and a longer half-life (103). Importantly, doxycycline hyclate (Periostat®) is the only MMPI approved for clinical use, although this is limited to periodontal disease. Of the 10 CMT analogues, CMT-3 (also called Col-3 or Metastat), has been effective in prostate cancer models of invasion and proliferation (117, 118), and is now in phase I clinical trials (119).

Despite significant intellectual and financial effort, progress in this area has been slow. Developing these drugs has been hindered by significant production/purification costs, short in vivo half-lives, and difficulty in delivering compounds to the target tissues in the joints (73). Another problem is the substantial redundancy among the MMPs in substrate specificity, suggesting that inhibiting a single MMP will not be therapeutically effective. Consequently, there is continuous debate about whether drugs should target several MMPs or whether multiple drugs should be used, each of which is directed at a single MMP (73). However, as NMR and X-ray crystallography increase our understanding of the structure and function of the various MMP active sites, this information is used to develop new classes of MMPI inhibitors. Some of these nontraditional MMPI compounds are under initial investigation and utilize functional groups such as bisphosphonates (120), thiol-related compounds (121), and coumadin derivatives (110).

7.3. Inhibitors of MMP Synthesis

Another mechanism for preventing MMP-mediated cartilage degradation is blocking gene expression (60, 61, 68). Theoretically, a decrease in MMP mRNA can be achieved through targeting the promoter region of the relevant MMP. Here too, however, there are potential problems with specificity, since the promoters of many MMPs share a similar structure and are often coordinately regulated. The one class of compounds in use clinically that inhibits MMP gene expression are the glucocorticoids (122). They act by binding to nuclear hormone receptors,

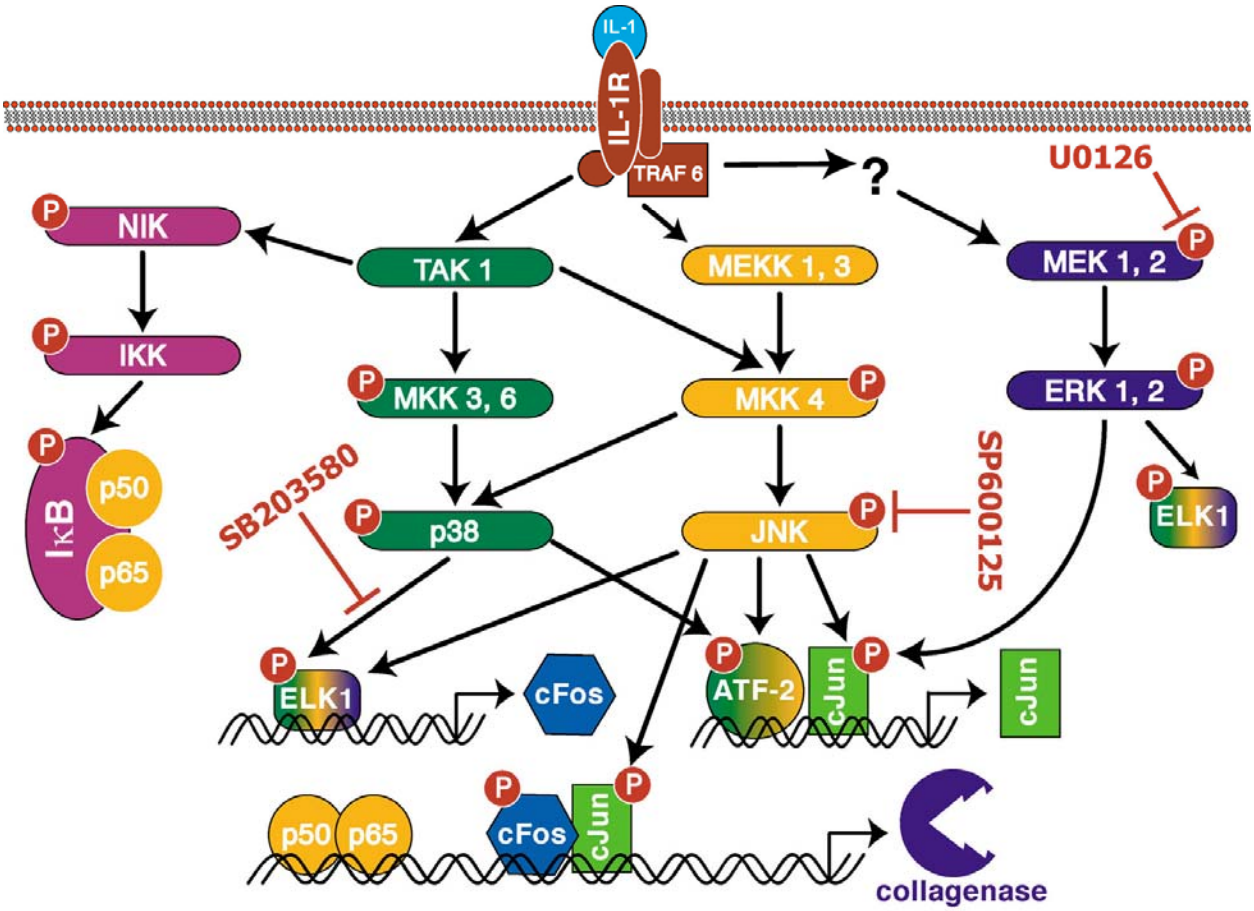

Figure 3. Major signaling pathways for IL-1 beta in chondrocytes and synovial cells. U0126 and SP600125 are chemical inhibitors that prevent the phosphorylation of MEK1, 2 and JNK, respectively. SB203580 is a chemical inhibitor known to block the kinase activity of p38.

which bind directly to DNA, or by binding to other transcription factors, leading to an indirect interaction with the DNA (18, 76). However, corticosteroids affect the expression of numerous genes, and consequently numerous side effects are associated with the chronic use of these drugs.

Vitamin A analogues are another class of compounds that inhibit MMP gene expression. Like the glucocorticoid hormones, these ligands also bind to nuclear hormone receptors (123-125). Specifically, all-trans and 13-cis retinoic acid and the synthetic retinoid, 4-hydroxyphenylretinamide, were all effective in reducing joint destruction in several animal models of arthritis. Importantly, retinoids and glucocorticoids were synergistic in their ability to inhibit MMP gene expression, suggesting that they could be given together in low doses that would selectively block MMP production. Unfortunately, the teratogenic side effects associated with retinoids have precluded their use as therapeutic compounds in arthritis. More recently, however, a novel retinoid has selectively inhibited the expression of genes with collagenolytic activity in cultured cells and in an animal model of arthritis (123, 124). Perhaps future studies will exploit the possible synergy with vitamin D analogues or glucocorticoids, with targeted inhibition of particular MMP genes, but without the toxicities associated with pan-retinoid agents.

The signal transduction pathways that regulate MMP gene expression are also potential targets for arthritis therapies (Figure 3). The signal transduction pathways activated when IL-1 beta and TNF-alpha bind to their cognate receptors on synovial cells and chondrocytes are potential drug targets. Chemically blocking MAPK pathways inhibits expression of MMP genes in tissue culture experiments, and also blocks the progression of arthritis in animal models. SB203580, a p38 MAPK inhibitor, blocks both MMP-13 gene expression in cultured chondrocytes (126), and IL-1 mediated collagen degradation in cartilage explants (127). In the collagen-induced arthritis model of rheumatoid arthritis, SB203580 inhibited the synthesis of TNF-alpha and IL-6, reduced paw inflammation, and reduced the joint destruction, thereby implicating p38 in the pathogenesis of arthritis (128). Importantly, orally active p38 inhibitors were also effective in animal models of inflammatory arthritis (129, 130), presumably by blocking MMP synthesis. The MAPK c-Jun n-terminal kinase (JNK) also contributes to MMP

regulation; JNK is necessary for MMP-13 induction in both chondrocytes and synoviocytes (126, 131), and inhibiting JNK with the chemical compound, SP600125, inhibited bone destruction in adjuvant induced arthritis (131). However, these regulatory points may be shared by several MMPs, and as a result the inhibition may not always be specific.

The NF-kB signaling pathway is a major pathway in controlling MMP gene expression, and is, therefore, a potentially important target (Figure 3). Not only does this pathway control the expression of several MMPs, it also regulates numerous inducible inflammatory genes (e.g., IL-1 beta, IL-6 and TNF-alpha) (63, 126, 132, 133). The importance of NF-kB in arthritis has been demonstrated with animal models where mice deficient for the p50 subunit were refractory to collagen induced arthritis (134). Thus, blocking the activity of NF-kB may be important therapeutically (63, 126, 135-137).

Interestingly, novel compounds that block the gene expression of specific MMPs are beginning to appear. One compound that may be useful therapeutically is a synthetic triterpenoid, 2-cyano-3,12-dioxoolean-1,9,dien-28-oic acid (CDDO) (138, 139). At nanomolar concentrations, CDDO selectively inhibited the transcriptional induction of MMP-1 and MMP-13 by inflammatory cytokines in cultured human chondrosarcoma cells and in rabbit articular chondrocytes, without affecting basal expression (138). The ability of CDDO to specifically regulate certain MMPs may avoid the side effects associated with broad spectrum inhibitors of MMP gene expression. CDDO has also shown anti-inflammatory activity through the inhibition of iNOS and COX-2 expression (139), suggesting that it may also target genes involved in the inflammatory response.

Importantly, direct blockade of inflammatory cytokines reduces MMP gene expression. Therapies targeted at TNF-alpha have documented the success of this approach for the treatment of RA. Since these drugs reduce inflammatory cytokines, they also decrease MMP synthesis, thereby decreasing joint destruction. Etanercept is a fusion protein of the ligand-binding region of the TNF-alpha receptor, which has been linked to the Fc portion of human IgG1 (140). Therapy with this drug reduced pain and inflammation as well as radiographic damage in RA, implying that MMP-mediated bone destruction was reduced (140). Infliximab is a chimeric monoclonal antibody against TNF-alpha (141), and when infliximab was used in combination with the classical drug, methotrexate (MTX), there was a chondroprotective effect that was greater than with either drug alone (141). Finally, combination treatment of MTX with adalimumab, another anti-TNF-alpha monoclonal antibody, significantly decreased the time to reach a 20% improvement in disease activity (ACR20), and led to significant decreases in the serum levels of proMMP-1 and proMMP-3 (142).

8. PERSPECTIVE

Even though several MMPs are involved in connective tissue degradation in both RA and OA, the two collagenases, MMP-1 and MMP-13, are probably the major mediators of collagen degradation and the irreversible joint destruction. They are, therefore, the pre-eminent candidates as therapeutic targets. MMP-1 is the most abundantly expressed collagenase in cartilage and synovium. Further, in RA MMP-1 is mostly likely the first collagenase that is produced, since the disease originates in the synovial fibroblasts adjacent to the cartilage and since MMP-13 expression in fibroblasts is generally quite low (143, 144). Thus, agents that specifically target MMP-1 may be preferable in preventing joint destruction in RA, especially in the early stages of this disease.

In contrast, MMP-13 appears to be emerging as an important therapeutic target in OA (145). This enzyme has the greatest specificity for type II collagen and also degrades aggrecan (146). Importantly, MMP-13 is produced almost exclusively by chondrocytes and is upregulated in conditions of inflammation (147). Finally, MMP-13 appears to play a predominant role in the early stages of OA suggesting that its inhibition might prevent escalation of disease to the more fulminant inflammatory stages.

No matter which MMP is the therapeutic target, the concept of "polypharmacy", has long been used in treating arthritic diseases: several drugs have been used, each with a different target, i.e. inflammation, proliferation, joint destruction. In the past, these drugs have had broad based effects on many cells, such as methotrexate, glucocorticoids and even the NSAIDs. In the future, we may be able to apply our knowledge of the crystal structure of the MMPs and of the signal transduction pathways and their molecular targets so as to develop the idea of "molecular polypharmacy." This new approach would embody the concept in which several drugs are used together, each targeted at specific pathways or genes. Nonetheless, the signal-transduction pathways and the transcriptional and post-transcriptional mechanisms regulating MMP gene expression are complex and often inter-connected, underscoring the difficulties inherent in designing successful therapies. However, perhaps new therapies will take advantage of synergistic effects of particular compounds for particular targets so that an additional layer of specificity is achieved. As the era of molecular medicine in the 21st century continues to transform our treatment of serious diseases such as cancer and atherosclerosis, there is continued optimism that arthritic disease will benefit as well.

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