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DEGRADATIVE ENZYMES IN OSTEOARTHRITIS

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1. ABSTRACT

A central feature of the osteoarthritic disease process involves erosive destruction of the articular cartilage extracellular matrix (ECM) on the surfaces of diarthrotic joints. The resultant loss of joint function makes studies on mechanisms underlying ECM degradation critical for treatment of the disease and prevention of disability. Candidate pathways to account for the loss of cartilage involve expression of a combination of proteases that degrade the major cartilage matrix macromolecules, aggrecan and type II collagen. The specific types of enzymatic activities associated with the progressive removal of ECM and severity of joint disease include the matrix metalloproteinases, collagenase, gelatinase and aggrecanase(s). The degradative enzymes originate in synovial cells, cartilage cells, the chondrocytes, distributed within the ECM and leukocytes that actively invade the joint space. Specific enzymes arising from each of these tissues exhibit selective ECM degrading properties; the different categories of these tissue-derived enzymes will be discussed in this chapter. A perspective on the efficacy of existing agents and the potential for development of novel therapeutic agents is also included. While the degradative enzymes serve as a focal point for therapeutic intervention, a fundamental understanding of the mechanisms underlying degradative enzyme expression in osteoarthritis remains an important goal for prevention of disease.

2. INTRODUCTION

Osteoarthritis is a disease process with multiple etiologies that afflicts a majority of the population in the later decades of life. Some of the factors contributing to disease susceptibility include genetics, body mass, previous history of trauma to a major limb, occupational influences, and immobilization. The disease in all cases culminates in the stepwise degeneration of diarthrotic joint integrity and function. A principal cause of joint morbidity results from degradation of the articular cartilage extracellular matrix (ECM). Since the specialized articular cartilage ECM ensures distribution of mechanical loads generated by weight bearing, loss of function quickly follows ECM breakdown. The loss of function manifests primarily through painful and highly restricted joint movement. The impact of osteoarthritis on personal productivity and quality of life in an aging society is increasingly being recognized as an important element of health care costs. The end stage of osteoarthritis usually requires total joint replacement. The current total health care cost attributable to this procedure is estimated to be hundreds of thousands annually.

This review delineates the role of various candidate enzymes implicated as causative agents in the loss of joint function. The discussion will include a short perspective on inhibition of degradative enzyme activity as a target for therapeutic intervention. Disease processes impacted by enzymatic degradation of extracellular matrix are vast and the subject area represents a dynamic and expansive topic of current research in biology and medicine. The molecular mechanisms involved in ECM degradation encompass specialized investigations within such diverse fields as tissue differentiation, growth factor and cytokine biology, immunological cell selection models and tumor cell metastatic disease. To limit the central focus to joint disease, the subject matter covered will establish a catalog of enzymatic activities that are recognized as strong candidates in the destruction of cartilage ECM in osteoarthritis.

The information will be presented within a structural framework that is organized around the specific tissues thought to elaborate the degradative enzymes associated with the disease process. In this regard, three major tissues must be considered the synovium, the

articular cartilage and the immune lymphoid organs. The synovium is a thin tissue that lines the joint space and is the structure that defines the boundaries of the synovial cavity. However, when inflamed, the synovium contributes to joint degradation. The second tissue is articular cartilage. At first glance, the cartilage appears to be metabolically inactive and to serve solely as an inert weight-bearing tissue lining the bony surface. However, perturbation of the chondrocytes within the cartilage ECM results in the release and activation of enzymes capable of ECM degradation. The immune system is the third candidate as a source of the degradative enzymes that when stimulated, attack the cartilage ECM macromolecules.

Therapeutic agents recognized as efficacious in blocking the degradative processes that involve cartilage degrading enzymes will be described to provide some perspective on treatment. Although no definitive answers are available with respect to prevention of osteoarthritis, continued discovery of the genetic, physical and metabolic influences on joint tissue metabolism predicts a promise of significant advances in our understanding of the role of degradative enzymes in osteoarthritis.

3. ARTICULAR CARTILAGE

The functional capability of articular cartilage rests with its three primary components, water, proteoglycan and type II collagen (1-5). The water content of cartilage depends largely on the proteoglycan content. The presence of water in association with the hydrophilic and negatively charged glycosaminoglycans contributes to the compressive resilience of the tissue. Articular cartilage proteoglycans include large and small proteoglycans with varying amounts of glycosaminoglycans and oligosaccharides covalently attached to a core protein. The proteoglycans and their constituent glycosaminoglycans originate as products of the chondrocyte. The proteoglycans are transported from the cells to the extracellular environment through multiple processing steps. The chondrocytes are non-randomly distributed throughout the cartilage matrix and exhibit variation in metabolism that coincides with location (6,7). Alteration of the chondrocyte morphology also modifies collagen synthesis (8) and may contribute to release of latent degradative enzymes such as procollagenase (9).

Type II collagen is assembled as cross-linked fibrils and provides cartilage with tensile strength (10,11). Collagen is protected from denaturation by the macromolecular complexes of aggrecans (large aggregating proteoglycans) (12-17). Aggrecans consist of a core protein having a molecular weight of 200-350 kilodaltons (18-20) to which the individual glycosaminoglycan (GAG) chains, chondroitin and keratan sulfate, are attached by covalent linkages to either a serine or threonine residue (21-23). The large proteoglycans contain approximately 100 chains of chondroitin sulfate, 40-50 keratan sulfate chains, 60-70 O-linked and 6-8 N-glycosidically linked oligosaccharide chains (24). Core proteins exhibit extensive homology (> 80%) for chicken, rat and human proteoglycan (25). A number of other minor but potentially structurally important molecules such as fibromodulin, types IX and XI collagen, decorin and cartilage oligomeric protein are interspersed within the major macromolecules, the aggrecans and type II collagen.

4. HISTORICAL VIEW

The early recognition of the extensive nature of the articular cartilage erosion from the bony surface makes the quest for the responsible agent a high priority. After years of consideration of lysosomal-like degradative enzymes, careful evaluation of lesions within human cartilage suggested a role for collagenase activity (26). In fact, studies of swelling pressure of osteoarthritic cartilage in vitro found that water content was increased. It was concluded that the increase in water content was due to a failure of the collagen network to resist expansion (27). A number of studies revealed that the joint space may remain in the neutral pH range and that proteases active at neutral pH may be significant to the degradative process (28,29). This consideration prompted a number of studies of ECM degradation to be carried out with isolated tissues.

Early studies screening degradative enzymes in homogenized cartilage collected from joints of animal models of osteoarthritis provide clues that neutral protease activity correlated with cartilage degradation. In many of the early studies, the index of degradation was the extent of the cartilage surface area showing fibrillation. Fibrillation was defined as a loss of integrity of the outer, smooth surface of the articular cartilage. The visualization of the extent of fibrillation often was achieved with particulate dye staining of the surface. Analysis of forty-nine specimens of osteoarthritic cartilage revealed increased collagenolytic activity and the highest levels of activity coincided with osteoarthritic lesions on the surface (30). Continued study of human osteoarthritic cartilage showed that proteolytic activity increased in proportion to the severity of disease and the significance of proteases as contributors to cartilage breakdown was increased by evidence that inhibiting the activity with chelators slowed the arthritic process (31). The role of the proteases active at neutral pH was extended to degradation of the proteoglycan. The effects of these enzymes on the proteoglycan became implicated in the loss of the ability of the large aggregating proteoglycans (aggrecans) to form stable non-covalently linked macromolecular complexes with hyaluronic acid. This supposition was strengthened by the demonstration that cleavage of the core protein released the hyaluronic acid binding domain from the remaining aggrecan structure (32). One important feature of the osteoarthritic cartilage was the discovery in a canine model that the degradation of the matrix was accompanied by changes in the character of the proteoglycans so that the chondroitin-sulfate chains were of a larger hydrodynamic size (33). This discovery presaged the recognition that some abortive reparative effort occurs within the ECM that may be associated with the onset of cell cloning.

5. SYNOVIAL ENZYMES

The distinction between what is a synovial-derived degradative enzyme versus a degradative enzyme

from other sources remains difficult to discern. One characteristic of osteoarthritis is that there is often evidence of synovitis at the margins of the joint but the role of synovial tissue in cartilage degradation remain unclear. In general, the floridity of the synovitis in osteoarthritis does not compare with that observed in an inflammatory disease such as rheumatoid arthritis. However, the difference in the two disease conditions permits some comparisons to be made.

Analysis of synovial fluid from an animal model of osteoarthritis showed no significant difference in the concentration of latent metalloproteinase between normal and experimental knees (34). In the same animal model, interleukin-6 levels were significantly elevated. In a model of slowly progressive osteoarthritis induced by a tibial valgus osteotomy, the synovial fluid of the operative knee exhibited increased levels of matrix metalloproteinase-3 (stromelysin) (35). At later stages of disease (18 months after surgery), the molar ratio of MMP-3 to tissue inhibitor of metalloproteinases-1 (TIMP-1) was higher in the operative knee. A comparison of the differential expression of cathepsins B and L in the synovial membrane of patients with rheumatoid arthritis and osteoarthritis revealed that matrix metalloproteinase mRNA expression was greater than cathepsin expression (36). The levels of cathepsin and metalloproteinase proteins were elevated in the rheumatoid synovial lining suggesting that post-transcriptional up-regulation of the enzymes occurred. Recent work suggests that the cathepsins may act to accelerate the degradation processes by adding to metalloproteinase activity (37). Comparison of MMP-3 levels synovial fluid of hips with osteonecrosis of the femoral head and hips with osteoarthrosis showed measurable levels of MMP-3 in both sources with levels being higher in the osteonecrosis samples (38). TIMP-1 levels were the same in both groups. Matrix metalloproteinase-13 was also measurable in the synovial stroma in samples collected from rheumatoid and osteoarthritic knees (39). The level of MMP-13 was higher in the rheumatoid synovium when compared to the osteoarthritic tissue.

An overwhelming amount of evidence from studies of synovial tissue supports the view that in chronic inflammatory conditions such as rheumatoid arthritis, synovial-derived degradative enzymes contribute to joint destruction. The same evidence suggests that synovial tissue in osteoarthritis exhibits some level of inflammatory enzymes such as MMP-3 but the synovium is not a major source of degradative activity. Such a conclusion leaves open the question of where the degradative enzymes arise in osteoarthritis. However, RT-PCR analysis of mRNA from cells from osteoarthritic synovial fluid showed the presence of matrix metalloproteinase-9 (MMP-9) signal (40).

6. CHONDROCYTE ENZYMES

Studies of normal and osteoarthritic cartilage under a variety of conditions ranging from characterization of human cartilage degradative enzymes from autopsy samples to *in situ* hybridization studies of cartilage from animal models of osteoarthritis implicate the chondrocyte in the destructive process. In normal articular cartilage, chondrocytes are metabolically active cells that are essentially non-dividing and which are primarily involved in the gradual turnover of the aggrecan components of the ECM. However, many studies have established that the chondrocyte is a cell in a state of “calm before a storm”. The storm being the process activated by acute phase reactants of the inflammatory process. The proinflammatory mediators, interleukin-1 alpha and beta, tumor necrosis factor alpha and other components such as transforming growth factor beta, phospholipase A2 and oxygen intermediates are capable of triggering the activation of latent neutral metalloproteinases (41-47). Once activated, the matrix metalloproteinases represent a group of enzymes that effectively degrade the cartilage ECM in a sudden and potentially irreversible manner (48-51).

A consideration of the degradative enzymes expressed by the articular chondrocyte fails to reveal a particular enzyme that stands out as singularly critical to the disease process. Some forms of the degradative enzymes appear to be selectively changed with respect to disease condition, time in the history of disease or at a particular location on the joint surface. A pragmatic approach would suggest that multiple enzymes likely play overlapping roles particularly as different ECM components serve as substrate (52). The latent metalloproteinases also represent substrate molecules as conversion of latent forms of these enzymes contributes to a progression in the degradative cascade.

In some respects, the degradative enzyme cascade active in cartilage degradation resembles other enzymatic cascades such as that represented by the complement system. The complexity of the activation process may require a redundant regulatory activation, involving post-transcriptional and post-translational mechanism, to ensure tissue and matrix stability during periods of excessive load, prolonged infection or massive trauma.

The enzymatic activities directed at the major matrix components, collagen and aggrecan, remain the focus of investigation of cartilage ECM degradation. The early studies on arthritic cartilage initially centered on the cathepsins but this emphasis was replaced following demonstration of collagenase in the matrix (53-57). Thereafter, a number of studies confirmed the expression of a variety of matrix metalloproteinases in cartilage. The forms that have been reported to be present in varying levels depending on the state of the cartilage, include MMP-9, MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-13 and membrane type 1 MMP (MT1-MMP) (58-65). RT-PCR studies of 54 osteoarthritic samples from twenty-four patients revealed that differential expression of MMP-9 mRNA coincides with severity of cartilage degradation (66). The severity of cartilage breakdown was determined by the extent of fibrillation of the surface and the mRNA expression was confirmed by *in situ*

hybridization. Importantly, this study revealed that MMP-9 expression was unregulated in osteoarthritic cartilage that exhibited a normal surface appearance. One hypothesis linking the expression of MMP-9 to inappropriate levels of mechanical loading involves the unregulated expression of the enzyme.

The role of increased collagenase activity on the cleavage of type II collagen was first suggested by the early studies confirming that active enzyme was extractable from osteoarthritic cartilage but not from normal articular cartilage (67). The role of the collagenases in the direct breakdown of type II collagen has been strengthened considerably. Immunohistological studies confirm an increased presence of neoepitopes in osteoarthritic cartilage that correspond to degraded fragments of a purified type II collagen substrate. The neoepitopes correspond to fragments produced by MMP-1 (collagenase-1), MMP-8 (collagenase-2) and MMP-13 (collagenase-3) (68). MMP-7 expression was localized to chondrocytes in the superficial and the transitional zones of osteoarthritic cartilage (69). The expression of MMP-7 showed a linear correlation with an increase in disease severity using the Mankin histological scoring system. Recent studies showed that expression of at least one member of a new family of proteins that exhibit a disintegrin-like and a metalloproteinase-like domain might also be involved in the pathobiology of osteoarthritis (70).

A central feature of the onset of cartilage degradation concerns the loss of the aggrecans from the extracellular matrix. As discussed above, the reduction of aggrecan content significantly alters the material properties of cartilage that provide much of its loading bearing function. In particular, loss of aggrecan changes both endogenous water content and the frictional resistance of water to leave the ECM. Changes in these parameters decrease compressive resilience and may contribute to disruption of the collagenous organization. Analytical studies of cartilage degradation products showed that the core protein of the aggrecans underwent cleavages that were consistent with matrix metalloproteinase specificity but also showed a unique peptide product suggestive of a different enzyme. This activity was attributed to a putative aggrecanase and more recent data implicated this enzyme in the osteoarthritic process (71). However, the fact that both the VDIPEN- and the NITEGE-neoepitopes produced from aggrecan are detected in joint cartilage indicates that multiple enzyme activities contribute to disease (72).

7. INFLAMMATORY CELL ENZYMES

The inflammatory cell enzymes include the metalloproteinases that may originate from the monocytes, macrophages and neutrophils that invade the synovial lining during inflammation. A study of MMP-9 expression in rheumatoid and inflammatory arthritis synovium showed that the leukocytes, neutrophils and macrophages, and the endothelial cells infiltrating the tissue expressed elevated levels when compared to synovial tissue from osteoarthritic joints (73). However, cells of synovial fluid samples aspirated from osteoarthritic joints exhibited significant MMP-9 expression.

The osteoarthritic joint may also exhibit an associated release of proinflammatory mediators such as interleukin-1 that may contribute to joint destruction by inducing the release of degradative enzymes (74). The activity of interleukin-1 as an inducer of cartilage matrix degradation is now understood to include induction of matrix metalloproteinase synthesis by chondrocyte and inhibition of the synthesis of the cartilage matrix components, type II collagen and aggrecan (75-77). Evidence exists that the effects of interleukin-1 on cartilage metabolism is less severe in immature cartilage when compared to cartilage obtained from animals having reached sexual maturity (78). The major age effect appeared to be the recovery of aggrecan synthesis following treatment with interleukin-1 *in vitro*. These data form the basis for considering that osteoarthritis exhibits a greater association with age because of the intrinsic susceptibility cartilage to respond to the proinflammatory cytokines. Other effects of interleukin-1 on joint tissue include an increase in chondrocyte expression of matrix metalloproteinase-9, which is elevated in the presence of protein kinase C activators (79). Interleukin-1 also increased the expression of phospholipase A2 in rabbit chondrocytes so that substrate availability for prostaglandin synthesis was increased in the joint (80).

8. HOMEOSTATIC MECHANISMS

The observation that cartilage remains intact and fully functional in some individuals over six to seven decades provides a strong argument for the existence of tightly coupled homeostatic mechanisms to ensure ECM stability. One major hypothesis addressing the longevity of ECM integrity states that a balance between levels of tissue inhibitors of matrix metalloproteinases (TIMP-1, TIMP-2, and TIMP-3) and matrix metalloproteinases (MMPs) prevents ECM degeneration (81,82). TIMP-2 was constitutively expressed by human chondrocytes and the level of expression was unchanged by serum, interleukin-1, interleukin-6 or transforming growth factor-beta. TIMP-1 expression by chondrocytes did respond to these agents (83). The expression of TIMP-3 in primary human and bovine articular chondrocytes was also increased by serum factors, including transforming growth factor-beta (84). These data suggest that differential regulation of the TIMP protein occurs in articular cartilage. A loss in regulatory factors may be responsible for an imbalance in the ratio of inhibitor to protease in osteoarthritic cartilage. A number of studies have confirmed that the relationship between these important proteins is perturbed with metalloproteinase activity being greater than TIMP levels (85-87).

Another regulatory process may involve the selective activation of the matrix metalloproteinases. A proenyzme activator of MMP-3 occurs in articular cartilage and results in the step-wise processing of the MMP-3 propeptide to generate multiple active forms of protease. The catalytic properties of the proenyzme activator share a resemblance to other metalloendopeptidases that exhibit specificity for single arginine or dibasic propeptide cleavage sites (88). Studies on membrane-type 1 MMP in

OA cartilage samples show that membranes of osteoarthritic chondrocytes can activate proMMP-2 which once activated exhibited a wide range of substrate specificity against components of the cartilage ECM (89,90)

9. PERSPECTIVE ON THERAPEUTIC AGENTS

The emphasis placed in this review on the role of metalloproteinases in cartilage matrix destruction emphasizes the need for therapeutic modalities capable of blocking their action. The involvement of the individual proteinases in the degradation of specific proteins makes the task a difficult one. The recognized role of inflammation in cartilage degradation has prompted a number of studies that evaluated agents such as the non-steroidal antiinflammatory drugs. In some instances, the NSAIDs exhibited efficacious effects on cartilage degeneration and intervention in the protease cascade (91,92). The drugs also showed some selective reactions with respect to chondrocyte metabolism (93). However, as a group, the matrix metalloproteinases remain of central interest as an appropriate target for preventing joint destruction in osteoarthritis.

Numerous studies show that members of the tetracycline family of antibiotics are effective in inhibiting collagenase and gelatinase activity (94-96). Oral administration of the one of these agents, doxycycline, proved to decrease both collagenase and gelatinase activity in cartilage from endstage hip osteoarthritis (97). These data suggest that an effective oral dose of doxycycline may be tolerated in a clinical trial to assess efficacious effects on cartilage degradation in osteoarthritic patients.

Other efforts to address the effects of enzymes such as collagenase and stromelysin on the extracellular matrix of cartilage have focused on synthetic compounds that can inhibit the enzymatic activity (98-100). This group of compounds includes chelating agents targeted to the metal dependency of the enzymes and molecules that are active site inhibitors and other agents that block enzyme synthesis. The success in the therapeutic arena has been complicated by the multifactorial nature of the disease process itself (101). A single effector molecule may only decrease one element in the cascade of degradative steps. If that step is early in the process of conversion of latent protein to active protein, the efficacy of the agent in question will be improved.

The recognition of the role of proinflammatory cytokines to the pattern of expression of cartilage degrading enzymes has resulted in a number of approaches to effectively counter their action. As a result, promising data are available that sequestration of proinflammatory agents by molecules such as interleukin-1 receptor antagonist (102,103,104) and antibody to tumor necrosis factor-alpha (105,106,107) can modulate progression of disease. Application of sets of anti-inflammatory cytokines could also intervene in the progression of osteoarthritis by countering the local synovitis associated with osteoarthritis (108,109).

Significant interest lies in the use of genetic methods to redirect tissue metabolism to offset the degradative cascade in joint (110,111). A number of efforts have already been directed to transfection of synovial cells as a means of regulating proinflammatory cytokine activity (112,113). These types of approaches will likely be extended to efforts to modulate degradative enzyme expression as well. Such a metabolic approach may permit a systematic block in the activation of cartilage degrading enzymes through the control to the intermediate steps in the processing of the latent to active enzyme.

10. CONCLUSIONS

The prevention of cartilage degradation in osteoarthritis remains a goal for clinician and scientists alike. The multitude of factors that initiate of the breakdown of the cartilage matrix will always remain a threat to normal joint function. Altering the character of the proteins composing the ECM will only be appropriate when the material properties of the ECM that permit functional load distribution can be preserved. In contrast, a fundamental understanding of the degradative enzymes that contribute to the ECM degradation and the mechanisms by which enzyme activation occurs will permit strategies for preserving joint function. The outcome of regulation of cartilage ECM degradation will be a significant reduction in patient morbidity and increased personal productivity. The degradative enzymes and tissues that may contribute the enzymes presented in this review provide guideposts for directing efforts to control cartilage degradation in osteoarthritis.

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